

**TOIDU JA LOOMASÖÖTADE MIKROBIOLOOGIA**  
**Horisontaalmeetodid *Enterobacteriaceae* avastamiseks ja**  
**arvuliseks määramiseks**  
**Osa 1: *Enterobacteriaceae* avastamine ja arvuline**  
**määramine eelrikastusega MPN meetodiga**

**Microbiology of food and animal feeding stuffs**  
**Horizontal methods for the detection and enumeration of**  
***Enterobacteriaceae***  
**Part 1: Detection and enumeration by MPN technique with**  
**pre-enrichment**  
**(ISO 21528-1:2004)**

**EESTI STANDARDI EESSÕNA****NATIONAL FOREWORD**

<p>See Eesti standard EVS-ISO 21528-1:2011 „Toidu ja loomasöötade mikrobioloogia. Horisontaalmeetodid <i>Enterobacteriaceae</i> avastamiseks ja arvuliseks määramiseks. Osa 1: <i>Enterobacteriaceae</i> avastamine ja arvuline määramine eelrikastusega MPN meetodiga“ sisaldab rahvusvahelise standardi ISO 21528-1:2004 „Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of <i>Enterobacteriaceae</i> - Part 1: Detection and enumeration by MPN technique with pre-enrichment“ identset ingliskeelset teksti.</p> <p>Standard EVS-ISO 21528-1:2011 on jõustunud sellekohase teate avaldamisega EVS Teataja 2011. aasta detsembrikuu numbris.</p> <p>Standard on kätesaadav Eesti Standardikeskusest.</p>	<p>This Estonian Standard EVS-ISO 21528-1:2011 consists of the identical English text of the International Standard ISO 21528-1:2004 „Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of <i>Enterobacteriaceae</i> - Part 1: Detection and enumeration by MPN technique with pre-enrichment“.</p> <p>This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.</p> <p>The standard is available from the Estonian Centre for Standardisation.</p>
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**Käsitlusala**

See ISO 21528 osa määratleb eelrikastusega meetodi *Enterobacteriaceae* määramiseks. See on rakendatav:

- inimtoiduks ja loomasöödaks ettenähtud toodetele ja
- toidu tootmise ja toidu käitlemise valdkonna keskkonnaproovidele.

Arvulisel määramisel arvutatakse kõige töenäosem arv (MPN) pärast vedelas söötmes inkubeerimist temperatuuril 37 °C (või 30 °C)<sup>1)</sup>.

Seda meetodit rakendatakse:

- kui otsitavate mikroorganismide puhul eeldatakse kasvuvõime taastamise vajadust enne rikastust ja
- kui otsitav arv eeldatakse olevat vahemikus 1 kuni 100 milliliitri või grammi katseproovi kohta.

Selle ISO 21528 osa rakendatavuse piirang on tingitud meetodi tundlikkuse suurest varieerumisest (vt peatükk 11).

<sup>1)</sup> Üldiselt kasutatakse temperatuuri 37 °C, kui *Enterobacteriaceae* arvu loendatakse hügieeni indikaatorina. Alternatiivina võib valida temperatuuri 30 °C, kui *Enterobacteriaceae* loendatakse tehnoloogilistel eesmärkidel ja see hõlmab psühhotroopset *Enterobacteriaceae*'d

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## Introduction

This part of ISO 21528 is intended to provide general guidance for the examination of products not dealt with by existing International Standards and to be taken into account by organizations preparing microbiological test methods for application to foods or animal feeding stuffs. Because of the large variety of products within this field of application, these guidelines may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply the guidelines provided as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this part of ISO 21528 is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviation from them in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this part of ISO 21528 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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# **Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae —**

## **Part 1: Detection and enumeration by MPN technique with pre-enrichment**

### **1 Scope**

This part of ISO 21528 specifies a method, with pre-enrichment, for the detection of Enterobacteriaceae. It is applicable to

- products intended for human consumption and the feeding of animals, and
- environmental samples in the area of food production and food handling.

Enumeration is carried out by calculation of the most probable number (MPN) after incubation at 37 °C (or 30 °C<sup>1)</sup>) in liquid medium.

This method is applicable

- when the microorganisms sought are expected to need resuscitation before enrichment, and
- when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.

A limitation on the applicability of this part of ISO 21528 is imposed by the susceptibility of the method to a large degree of variability (see Clause 11).

### **2 Normative references**

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1:1999, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

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1) The temperature of 37 °C is generally used when the enumeration of Enterobacteriaceae is for a hygienic indicator. Alternatively, a temperature of 30 °C can be chosen when the enumeration of Enterobacteriaceae is conducted for technological purposes and includes psychrotrophic Enterobacteriaceae.

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

### 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

#### 3.1

##### **Enterobacteriaceae**

microorganisms that form characteristic colonies on violet red bile glucose agar and that ferment glucose and show a negative oxidase reaction when the tests are carried out in accordance with the methods specified in this part of ISO 21528

#### 3.2

##### **detection of Enterobacteriaceae**

determination of the presence or absence of these bacteria, in a particular quantity of product, when tests are carried out in accordance with this part of ISO 21528

#### 3.3

##### **enumeration of Enterobacteriaceae**

most probable number of Enterobacteriaceae found per millilitre or per gram of the test sample when the test is carried out according to the method specified in this part of ISO 21528

### 4 Principle

#### 4.1 Detection of Enterobacteriaceae (see Annex A)

##### 4.1.1 Pre-enrichment in non-selective medium

Buffered peptone water (BPW) is inoculated with the test portion, then incubated at 37 °C (or 30 °C)<sup>1)</sup> for 18 h ± 2 h.